# TOTAL-FIX®

Comments by Lynne Garcia

Although the formula for TOTAL-FIX® is proprietary, it does not contain any mercury, formalin, or polyvinyl alcohol (PVA). The TOTAL-FIX® stool collection kit is a single vial system that provides a standardized method for untrained personnel to properly collect and preserve stool specimens for the detection of helminth larvae and eggs, protozoan trophozoites and cysts, coccidian oocysts, and microsporidian spores. Fecal concentrations, permanent stains, and most fecal immunoassays can be performed from a TOTAL-FIX® preserved specimen. TOTAL-FIX® preserves parasite morphology and helps with disposal and monitoring problems related to mercury and formalin encountered by laboratories. The following questions/answers may be helpful for the user.

**THE KEY QUESTION IS NOT HOW BEAUTIFUL THE ORGANISMS ARE, BUT CAN YOU TELL WHICH ORGANISMS ARE PRESENT! WITH THE EXCEPTION OF VERY RARE ORGANISMS SUCH AS *E. NANA* CYSTS, GENERALLY ALL ORGANISMS THAT CAN BE IDENTIFIED USING MERCURY FIXATIVES CAN ALSO BE IDENTIFIED USING TOTAL-FIX.**

1. **What is TOTAL-FIX®?**
   TOTAL-FIX® is a fixative with a proprietary formula, it is similar to Unifix and Zinc PVA (Z-PVA), commonly used fixatives that have been commercially available and used in many laboratories since 1992.

2. **What is PVA?**
   PVA stands for polyvinyl alcohol, a plastic powder/resin that is incorporated into the fixative (Schaudinn's or other fixatives such as Z-PVA) and serves as an adhesive to "glue" the stool material onto the slide. **PVA itself has no preservation capability.**

3. **Since PVA is used to "glue" the stool onto the slide prior to permanent staining, how does the stool preserved in TOTAL-FIX® stick to the glass slide?**
   The TOTAL-FIX® user can add some albumin to the slide prior to preparation of the stool smear for staining. **HOWEVER, IF THE STOOL SMEAR IS THOROUGHLY DRY PRIOR TO STAINING, ALBUMIN WILL NOT BE NECESSARY.** See TOTAL-FIX® algorithm.

4. **What material should be used from the TOTAL-FIX vial containing stool for preparation of the smears for permanent staining?**
   Centrifuge some of the TOTAL-FIX®/Stool mixture for 10 min at 500 xg (DO NOT USE ANY RINSE SOLUTIONS – FORMALIN, SALINE, or WATER). Pour off the excess fixative after the centrifugation and use the sediment to prepare the fecal smears for permanent staining.

**NOTE**: **You can now add a rinse solution and proceed with the routine concentration.**

4 **How long should the slides prepared from TOTAL-FIX® be dried prior to processing for routine staining (trichrome or iron-hematoxylin)?**
Spread the sample over the slide to prepare a thin smear which varies in thickness (routine preparation of slides – no changes required). Allow to dry overnight at room temperature or for several hours (minimum of 30 min; 60 min if slide is thicker) in a 37°C incubator or slide warmer (the smear will appear opaque when dry). Many labs routinely prepare their smears and then place the smears on a tray in the incubator (37ºC) for 60 min to dry. The fecal smears can be left longer in the incubator with no damage at all to the slides. Do not use a heating block; the higher temperature for most heating blocks will be detrimental to any organisms (amebae, flagellates, coccidia, microsporidia) present.

5. **What should *Giardia lamblia* and other protozoa look like when stained from normal patient clinical specimens (no lag time between specimen passage and fixation)?**
Organism morphology should appear no different from organisms preserved in Z-PVA, with good overall morphology for trophozoites, cysts (routine trichrome or iron-hematoxylin), coccidian oocysts (modified acid-fast stains), and microsporidial spores (modified Trichrome).

6. **Is there any difference in the overall organism staining with and without PVA? Since PVA is a plastic powder, when placed in a fixative solution, there may be some background staining, particularly if the PVA has not been absorbed using paper towels prior to fecal smear preparation. Therefore, since TOTAL-FIX® does not contain PVA, the overall background stain tends to be more clean and precise in terms of morphology.**

## ALGORITHM FOR THE USE OF TOTAL-FIX® (UNIVERSAL FIXATIVE)

**(1) SPECIMEN VIAL CONTAINING TOTAL- FIX AND FECAL SPECIMAN**
(Ratio of 1/3 stool, 2/3 fixative – MIX WELL)
(Allow to fix 30 min before processing)

**(2) IMMUNOASSAYS**
Use liquid at top of vial to run immunoassays and cartridge tests for *Giardia* and *Cryptosporidium* (antigen) in the solution. If vial is shaken prior to testing let the particulate matter settle out before taking specimen from the top.

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**(3) TUBE OR CONCENTRATION DEVICE**
(Add approximately 1-2 ml fixative/specimen mix to the tube or device)
(Mix well prior to pouring into the tube or device)
(DO NOT ADD ANY RINSE FLUIDS: Saline, water, formalin)
**(Rinse fluids will prevent routine permanent staining.**

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**(4) CENTRIFUGE (10 min at 500 Xg)**

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**(5) POUR OFF MOST OF EXCESS FIXATIVE**

**(6) MIX SEDIMENT WITH REMAINING FIXATIVE**

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**(7) PREPARE SLIDES FOR PERMANENT STAINS/DFA; STAIN SLIDES**
(Allow slides to dry for ~30-45 min at 37ºC)
(Slides can be placed on trays and put in the incubator)
(Room temperature drying requires ~60 min)

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**(8) ADD RINSE FLUID TO REMAINING STOOL SEDIMENT (FROM STEP 5)**
(Mix sediment and rinse fluid well before centrifugation)
**PROCEED WITH ROUTINE CONCENTRATION PROCEDURE**
(Single rinse including Ethyl Acetate or Hemo-De step is recommended)
(DO NOT ADD Ethyl Acetate if the specimen contains a lot of mucus)

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**(9) RING DEBRIS LAYER, POUR OFF EXCESS FLUID**
MIX AND EXAMINE SEDIMENT AS CONCENTRATION SEDIMENT SLIDE
Entire 22 x 22mm coverslip: low power (10X objective)
1/3 – 1/2 coverslip: high dry power (40X objective)

# TOTAL-FIX® VALIDATION STUDIES FOR THE ROUTINE LABORATORY

## INTRODUCTION

The approach to validation of TOTAL-FIX® is based on the user's current fecal fixative. Various fecal fixatives and the recommended validation approach are indicated below. See also Table 1.

## QUALITY CONTROL SPECIMEN USED FOR VALIDATION STUDIES

Fixatives for fecal specimens are checked for quality control by the manufacturer before sale, generally with the use of living protozoa. If you prepare your own fixatives, the following approach can be used for quality control. The specimen used for quality control presented below is designed to be used with fixatives from which permanent stained smears will be prepared (Universal Fixatives, Schaudinn's fluid, fixatives with/without PVA, fixatives with a mercury base, fixatives with a zinc base, SAF, or MIF). The same quality control specimen can also be used in a concentration; the white blood cells (WBCs) can be seen in the concentrate sediment (sedimentation concentration) or in the surface film (flotation concentration).

**NOTE: Some have also used ATCC organisms as QC/validation studies (protozoa) or positive animal specimens; both are acceptable.**

1. Add approximately 2 g (size of 2 medium-large grapes) of soft, fresh fecal specimen (normal stool, containing no parasites) to a vial of the fixative(s) being tested. MIX WELL.

2. Obtain a fresh, anticoagulated blood specimen (lavender top, EDTA, 7 ml specimen), centrifuge, and obtain a buffy coat sample (try and find a specimen with a high WBC count).

3. Add the buffy coat specimen to the fixative/stool mixture (TOTAL-FIX®/stool) and the second fixative to be tested (your current fecal collection vial). MIX WELL.

4. Allow 30 min for fixation, and then prepare several fecal smears (as you normally would). Allow the smears to dry thoroughly (60 min) at room temperature or 30 min in an incubator (approximately 35°C). Do NOT use a heat block, and do not make the smears too thick.

5. Stain the slides by the normal staining procedure (trichrome, iron-hematoxylin).

6. After staining, if the WBCs appear well fixed and exhibit typical nuclear and cytoplasmic color and morphology, one can assume that any intestinal protozoa placed in the same lot number of preservative would also be well fixed, provided that the fecal sample was fresh and fixed within the recommended time limits.

7. This bulk quality control specimen can be concentrated as for a normal patient specimen. If the fixative is performing correctly, the WBCs will be visible in the concentration sediment or surface film (depending on the method used).

8. Record all quality control results. If the WBC morphology does not confirm good fixation, describe the results and indicate what corrective action procedures were used (repeated the test, prepared new fixative).

## TABLE FOR VALIDATION STUDY (PERMANENT STAINED FECAL SMEAR)

| STEP | PROTOCOL | # SPECIMENS<br># SLIDES | COMMENTS |
|---|---|---|---|
| **Step 1** | Prepare Quality Control specimen as indicated above, using three different stool specimens and three different buffy coat layers | 3 different specimens (stools, EDTA) | You will have 3 vials of TOTAL-FIX® QC and 3 vials of your regular fixative QC |
| **Step 2** | Prepare and stain 2 slides from each of the six vials<br><br>6 slides from TOTAL-FIX®<br><br>6 slides from your regular fixative | 6 slides from the TOTAL-FIX® vials and 6 from your regular fixative QC vial | You will have a total of 12 slides for routine staining (trichrome or iron-hematoxylin) |
| **Step 3** | Examine stained smears and record results (descriptions of WBCs); nuclear and cytoplasmic colors; lobed nuclei, any granules if present (eosinophils, or basophils), and overall morphology | 12 smears total | Have 2 different people examine the slides for comparison |

**NOTE: If your regular fixative is Unifix or Zinc PVA (Z-PVA) (Medical Chemical Corporation), the proprietary formulations are essentially the same and removal of the inert PVA plastic powder has no impact on organism morphology. In this case, validation would be**

**at the discretion of the user, but should not be required. No organism differences are seen in fecal concentrations (larvae, eggs, oocysts, spores).**

**The removal of PVA will provide a clearer fecal smear with less background haze, thus making microscope examination easier than before.**

**REMEMBER, THE FECAL SMEARS MUST BE THOROUGHLY DRY PRIOR TO STAINING.**

**A. Room temperature – 60 min or overnight**

**B. Incubator (standard bacteriology incubator): 30-60+ min; drying beyond 60 min will not be detrimental to the smears**

## TABLE FOR VALIDATION STUDY (FECAL IMMUNOASSAY)

| STEP | PROTOCOL | # SPECIMENS # TESTS | COMMENTS |
|---|---|---|---|
| **Step 1** | Save positive fecal specimens (*Giardia* and/or *Cryptosporidium*) for fecal immunoassay(s) (EIA, FA, or rapid cartridge formats) | 3 different specimens | You will have 3 vials of your regular fixative that are positive |
| **Step 2** | Obtain positive TOTAL-FIX® fecal specimens (*Giardia* and/or *Cryptosporidium*) for fecal immunoassay(s) (EIA, FA, or rapid cartridge formats) | 3 different specimens | You will have 3 vials of TOTAL-FIX® fixative that are positive |
| **Step 3** | Perform duplicate runs using your regular fecal immunoassay format from the 3 vials (current fixative – known positives) and the 3 vials (TOTAL-FIX® – known positives) | Record results for the duplicate runs on the 6 positive vials: total of 12 IA results | Have 2 different people perform the testing (one per run of 6 tests, the other per run of the 6 duplicates) |

# TOTAL-FIX® FIXATIVE AND COMPATIBILITY WITH VARIOUS IMMUNOASSAYS

The TOTAL-FIX® (Patent Pending) fixative is manufactured by Medical Chemical Corporation and is a single vial system for the preservation of fecal specimens. TOTAL-FIX®-preserved fecal specimens can be used for fecal concentrations, permanent stained smears (trichrome or iron-hematoxylin; special stains for coccidia and microsporidia), and certain fecal immunoassays for *Cryptosporidium* spp. and *Giardia lamblia*. TOTAL-FIX® (2807-05, 100 vials/case) is available from Medical Chemical Corporation. ***If the vial is shaken prior to testing, allow the large fecal debris to settle out before taking the specimen for immunoassay testing.**

| Company, Product | Compatibility Status | Comments* |
|---|---|---|
| Medical Chemical Corp. | | |
| PARA-TECT Giardia EIA | Compatible with TOTAL-FIX® | Use clear liquid from the top of the vial (antigen in solution) |
| PARA-TECT Cryptosporidium EIA | Compatible with TOTAL-FIX® | Use clear liquid from the top of the vial (antigen in solution) |
| PARA-TECT Giardia/Cryptosporidium DFA | Compatible with TOTAL-FIX® | Perform test on concentrate sediment (specimen rinsed with saline or formalin) |
| Remel | | |
| Xpect Cryptosporidium RAPID | Compatible with TOTAL-FIX® | Use clear liquid from the top of the vial (antigen in solution) |
| Xpect Giardia RAPID | Compatible with TOTAL-FIX® | Use clear liquid from the top of the vial (antigen in solution) |
| Xpect Giardia/Cryptosporidium | Compatible with | Use clear liquid from the top of the |

| | | |
|---|---|---|
| RAPID | TOTAL-FIX® | vial (antigen in solution) |
| ProSpect (EIA) Cryptosporidium | Compatible with TOTAL-FIX® | Use clear liquid from the top of the vial (antigen in solution) |
| ProSpect (EIA) Giardia | Compatible with TOTAL-FIX® | Use clear liquid from the top of the vial (antigen in solution) |
| Meridian Biosciences | | |
| ImmunoCard STAT Crypto/Giardia RAPID | Compatible with TOTAL-FIX® | Use clear liquid from the top of the vial (antigen in solution) |
| MERIFLUOR Cryptosporidium/Giardia DFA | Compatible with TOTAL-FIX® | Perform test on concentrate sediment (specimen rinsed with saline or formalin) |
| TechLab | | |
| Cryptosporidium II EIA | Compatible with TOTAL-FIX® | Use clear liquid from the top of the vial (antigen in solution) |
| Giardia II EIA | Compatible with TOTAL-FIX® | Use clear liquid from the top of the vial (antigen in solution) |

| **Company, Product** | **Compatibility Status** | **Comments** |
|---|---|---|
| Remel | | |
| ProSpecT Entamoeba histolytica EIA | Fresh, frozen, or Cary Blair | Suspect this is still not compatible with TOTAL FIX®, as well as with other fixatives |
| TechLab | | |

| | | |
|---|---|---|
| Entamoeba histolytica II EIA<br><br>*Test for the Entamoeba histolytica/E. dispar group no longer available* | Fresh or frozen specimens | Suspect this is still not compatible with TOTAL-FIX®, as well as with other fixatives |
| **Biosite** | | |
| Triage Rapid Cartridge | Fresh or frozen specimens | Giardia, Cryptosporidium, Entamoeba histolytica/E. dispar |

## COMMENTS REGARDING PREPARATION OF FECAL SMEARS FOR PERMANENT STAINING [TRICHROME, IRON-HEMATOXYLIN, MODIFIED ACID-FAST (COCCIDIA), MODIFIED TRICHROME (MICROSPORIDIA)] FROM TOTAL-FIX®

Some of the fixatives that do not contain PVA (single vial systems that can be used for the O&P, as well as the fecal immunoassays, including TOTAL-FIX®), will also stick to the slides if the specimen is centrifuged and the slides prepared from the first centrifugation sediment (no rinse fluid used). Then, the full concentration with rinses can be performed. Also, these slides can be dried for two hours in the incubator or overnight at room temp. If prepared and dried using these recommendations, the stool will not fall off the slides during staining (even without PVA or albumin). Each laboratory can try the albumin vs no albumin approach to see if their method requires extra "glue" for adherence of the fecal material to the slide (like the approach used for sodium acetate-acetic acid-formalin (SAF). TOTAL FIX® does not contain PVA, so specimens collected in this fixative can also be used for fecal immunoassay testing (see table above).

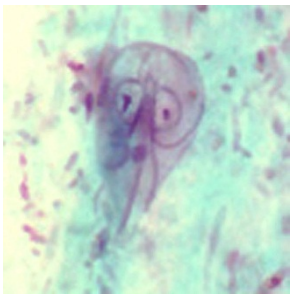
## ORGANISM MORPHOLOGY: COMPARISON OF MERCURY VS. NON-MERCURY FIXATIVES

The following sets of images will help to demonstrate some of the morphologic differences that may be seen using non-mercury (Hg) based fixatives compared with those using the mercuric chloride-based fixative. We are using the same organism to demonstrate the use of Hg and non-Hg fixatives on the organism morphology. Each laboratory may see some additional variations among their staining results. Although organism morphology may be excellent, often there is less detail visible when using the non-mercury based fixatives. Using mercury-based fixatives, there tends to be more room for error during the fixation process. In spite of the fact that the specimen may not be thoroughly mixed with the fixative, morphology is most often excellent. However,

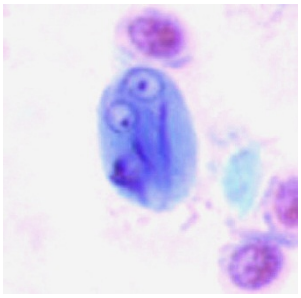
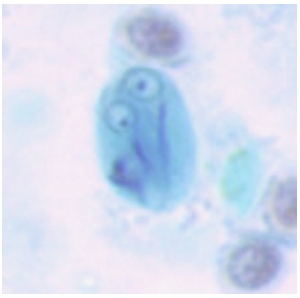
when inadequate mixing of specimen/fixative occurs using non-mercury based fixatives, the organism morphology may not be as good. Also, when using any fecal fixative, if the specimen is too old prior to fixation, organism morphology may be poor.

When using the Wheatley's modification of the trichrome stain, often the overall stain tends to contain more green tones with fewer blue and red tones when using non-mercury fixatives. However, the results may vary among different laboratories. Also, the choice of fixative/stain combination(s) will vary among laboratories.

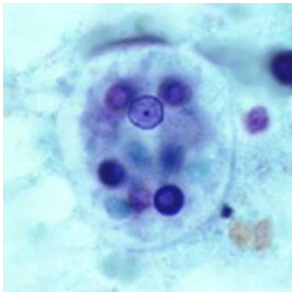
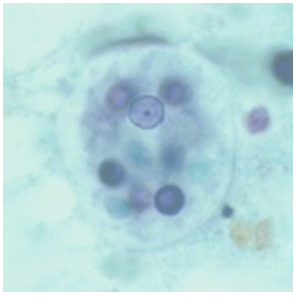
Hg Fixative Non-Hg Fixative




*Giardia lamblia* trophozoites

*Giardia lamblia* cysts

*Entamoeba histolytica* trophozoites

**NOTE: Since the non-mercury fixative is a "softer" fixative, some of the smaller cysts may be a bit more difficult to see if they are present in rare numbers; however, trophozoites tend to fix very well (no cyst walls), as do *Blastocystis hominis* forms. Overall, the organism morphology using TOTAL-FIX® is excellent, although non-mercury fixatives will never exactly match the quality seen using mercury. Again, the question is not how beautiful the organism is, but can it be identified. Using TOTAL-FIX®, the quality of organism morphology and identification have not been compromised.**